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Serial No.: 09/362,394  
Filed : July 28, 1999  
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Please amend the specification as follows:

On page 9, lines 14-35, please amend the paragraph as follows. A  
markedup version of the paragraph is attached hereto as Exhibit A.

C1  
--One of the applications of the present invention is the detection of the human hepatitis B virus surface antigen mutant 145 (Glycine to Arginine) using a solid glass supports device. In the present invention, further modifications have been added to two oligonucleotides (listed herein): 5'-TACGGACGGAAACT-3', and 5'-TACGGACAGAAACT-3', both located from position 582 to 595 as referred to the wild type human hepatitis B virus genome. These modifications include a fluorescent dye, 6-(fluorescein-6-carboxamido) hexanoate (6FAM), at its 5' terminus and a primary amine group at its 3' terminus. The resulting oligonucleotides that are immobilized on solid glass supports have the following structure: 5'-(6FAM)TACGGACGGAAACTGTTTTTTTTTTT (C-7 amine)-3', and 5'-(6FAM)TACGGACAGAAACTGTTTTTTTTTTT (C-7 amine)-3', and the second oligonucleotide contains the mutation G to A (position 8) leading to change at amino acid 145 (Glycine to Arginine) of human hepatitis B virus surface antigen. There is also an inclusion of a poly-T (underlined) as a synthetic linker aiming at facilitating the subsequent hybridization reaction with target human viral DNA sequences from serum samples.--

On page 12, lines 21-30, please amend the paragraph as follows. A  
marked up version of the paragraph is attached hereto as Exhibit A.

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C2 --For the novel detection system in the present invention, polymerase chain reaction is carried out using either plasmid DNA (containing coding region of either wild type or mutant 145 (Glycine to Arginine) of human hepatitis B virus surface antigen), or viral DNA as indicated in Figure 1. Oligonucleotides used in the said polymerase chain reaction are listed herein and have the following localization on the wild type human hepatitis B viral genome:--

On page 14, line 31 to page 15, line 15, please amend the paragraph as follows. A markedup version of the paragraph is attached hereto as Exhibit A.

C3 --As a direct application of the novel detection system in the present invention, modifications have been added to two oligonucleotides (listed herein): 5'-TACGGACGGAAACT-3', and 5'-TACGGACAGAAACT-3', both located from position 582 to 595 as referred to the wild type human hepatitis B virus genome. These include a fluorescent dye, 6-(fluorescein-6-carboxamido) hexanoate, at its 5' terminus for microscopic detection and a primary amine group at its 3' terminus allowing its immobilization on solid glass supports. The resulting oligonucleotides that are immobilized on solid glass supports has the following structure: 5'-(6FAM)TACGGACGGAAACTGTTTTTTTTTTT (C-7 amine)-3', and 5'-(6FAM)TACGGACAGAAACTGTTTTTTTTTTT (C-7 amine)-3', and the second oligonucleotide contains the mutation G to A (position 8 of the oligonucleotide, in bold) leading to change at amino

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C3  
acid 145 (Glycine to Arginine) of human hepatitis B virus surface antigen. There is also an inclusion of a poly-T (underlined) as a synthetic linker aiming at optimizing the subsequent hybridization reaction with target human viral DNA sequences from serum samples.--

In the claims:

Please cancel claims 1-18 without prejudice or disclaimer to applicants' rights to pursue the subject matter of these claims in a later-filed application and add new claims 19-68 as follows:

C4  
cont'd  
--19. (New) An oligonucleotide which (1) is immobilized, (2) comprises a sequence which corresponds to a portion of a wildtype human hepatitis B virus surface antigen nucleic acid, (3) is linked to a fluorescent dye at its 5' terminus, and (4) is linked to a primary amine group at its 3' terminus.--

Sub D3  
--20. (New) The oligonucleotide of claim 19, wherein the oligonucleotide comprises the sequence TACGGACGGAACTG.--

--21. (New) The oligonucleotide of claim 19, wherein the oligonucleotide comprises the sequence  
~~TACGGACGGAACTGTTTTTTTTTTT.~~

--22. (New) The oligonucleotide of claim 19, wherein the fluorescent dye is 6-(fluorescein-6-carboxamido) hexanoate.--

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--23. (New) The oligonucleotide of claim 19, wherein the primary amine group is a C-7 amine.--

--24. (New) The oligonucleotide of claim 19, wherein the oligonucleotide is immobilized on a solid support.--

--25. (New) The oligonucleotide of claim 24, wherein the solid support is a glass bead.--

Sub. 24  
24  
~~--26. (New) An oligonucleotide which (1) is immobilized, (2) comprises the sequence TACGGACGCAAACTGTTTTTTTTTTT, (3) is linked to 6-(fluorescein-6-carboxamido) hexanoate at its 5' terminus, and (4) is linked to a C-7 amine at its 3' terminus.--~~

--27. (New) An oligonucleotide which (1) is immobilized, (2) comprises a sequence which corresponds to a portion of a human hepatitis B virus nucleic acid, which portion comprises a mutation present in a mutant human hepatitis B virus, (3) is linked to a fluorescent dye at its 5' terminus, and (4) is linked to a primary amino group at its 3' terminus.--

--28. (New) The oligonucleotide of claim 27, wherein the mutation is present at the amino acid at position 145 of human hepatitis B virus surface antigen.--

~~--29. (New) The oligonucleotide of claim 27, wherein the oligonucleotide comprises the sequence TACGGACAGAACTG.--~~

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~~Sub D5~~  
~~30. (New) The oligonucleotide of claim 27, wherein the  
oligonucleotide comprises the sequence  
TACGGACAGAACTGTTTTTTTTTTT.--~~

~~31. (New) The oligonucleotide of claim 27, wherein the  
fluorescent dye is 6-(fluorescein-6-carboxamido)  
hexanoate.--~~

~~32. (New) The oligonucleotide of claim 27, wherein the  
primary amine group is a C-7 amine.--~~

~~33. (New) The oligonucleotide of claim 27, wherein the  
oligonucleotide is immobilized on a solid support.--~~

~~34. (New) The oligonucleotide of claim 33, wherein the solid  
support is a glass bead.--~~

~~35. (New) An oligonucleotide which (1) is immobilized; (2)  
comprises the sequence TACGGACAGAACTGTTTTTTTTTTT, (3)  
is linked to 6-(fluorescein-6-carboxamido) hexanoate at  
its 5' terminus, and (4) is linked to a C-7 amine at its  
3' terminus.--~~

~~36. (New) An oligonucleotide which (1) has a sequence which  
corresponds to a portion of a nucleic acid which encodes  
human hepatitis B virus surface antigen, and (2) is  
linked at its 5' terminus to a biotin group.--~~

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~~--37. (New) The oligonucleotide of claim 36, wherein the  
sequence is AGGATCAACAACAACAGTA.--~~

--38. (New) An oligonucleotide which (1) has a sequence which  
is complementary to a nucleic acid which encodes human  
hepatitis B virus surface antigen, and (2) is linked at  
its 5' terminus to a fluorescent dye.--

*Sub. D7*  
~~--39. (New) The oligonucleotide of claim 38, wherein the  
sequence is ATCGTCCTGGGCTTTGCAA.--~~

*C4*  
--40. (New) The oligonucleotide of claim 38, wherein the  
fluorescent dye is Texas red.--

*Sub. D8*  
~~--41. (New) The oligonucleotide of claim 38, wherein the  
sequence is ATCGTCCTGGGCTTTGCAA, and the fluorescent dye  
is Texas red.--~~

--42. (New) A composition which comprises two oligonucleotides,  
wherein:

- (a) the first oligonucleotide (1) has a sequence which  
corresponds to a portion of a nucleic acid which  
encodes human hepatitis B virus surface antigen,  
and (2) is linked at its 5' terminus to a biotin  
group; and
- (b) the second oligonucleotide (1) has a sequence which  
is complementary to a nucleic acid which encodes  
human hepatitis B virus surface antigen, and (2) is

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linked at its 5' terminus to a fluorescent dye.--

*Sub D9*  
--43. (New) The composition of claim 42, wherein (i) the first oligonucleotide has the sequence AGGATCAACAACAACAGTA; and (ii) the second oligonucleotide has the sequence ATCGTCCTGGGCTTTCGCAA, and the fluorescent dye is Texas red.--

*C4*  
--44. (New) A method for identifying a human hepatitis B virus surface antigen mutant 145 in a sample which comprises:  
(A) obtaining viral nucleic acid from a sample;  
(B) amplifying the viral nucleic acid in a polymerase chain reaction using two primers, wherein  
(1) one primer is an oligonucleotide which (i) has a sequence which corresponds to a portion of a nucleic acid which encodes human hepatitis B virus surface antigen, and (ii) is linked at its 5' terminus to a biotin group; and  
(2) the other primer is an oligonucleotide which (1) has a sequence which is complementary to a nucleic acid which encodes human hepatitis B virus surface antigen, and (2) is linked at its 5' terminus to a fluorescent dye;  
(C) obtaining, from the amplified nucleic acid, single stranded nucleic acid which comprises the fluorescent dye;  
(D) contacting the single stranded nucleic acid which comprises the fluorescent dye to an immobilized

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*Sub. D9*  
*C4*  
~~oligonucleotide, which oligonucleotide comprises a~~  
sequence which (i) corresponds to a portion of a  
human hepatitis B virus surface antigen nucleic  
acid, which portion comprises a mutation present in  
a mutant human hepatitis B virus, (ii) is linked to  
a fluorescent dye at its 5' terminus; and (iii) is  
linked to a primary amine group at its 3' terminus,  
under conditions permitting hybridization between  
the single stranded nucleic acid which comprises  
the fluorescent dye and the oligonucleotide,  
wherein hybridization between the single stranded  
nucleic acid which comprises the fluorescent dye and the  
immobilized oligonucleotide identifies the sample as one  
containing a human hepatitis B virus surface antigen  
mutant 145.---

--45. (New) The method of claim 44, wherein the mutation in  
step (D) (i) is present at the amino acid at position 145  
of human hepatitis B virus surface antigen.--

*Sub. D10*  
--46. (New) The method of claim 44, wherein the oligonucleotide  
comprises the sequence TACGGACAGAAACTG.---

--47. (New) The method of claim 44, wherein the oligonucleotide  
comprises the sequence TACGGACAGAAACTGTTTTTTTTTTT.---

--48. (New) The method of claim 44, wherein the fluorescent dye  
which is linked to the oligonucleotide is 6-(fluorescein-  
6-carboxamido) hexanoate.--



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*Sub D10*  
~~--49. (New) The method of claim 44, wherein the primary amine group which is linked to the oligonucleotide is a C-7 amine.--~~

~~--50. (New) The method of claim 44, wherein the oligonucleotide is immobilized on a solid support.--~~

--51. (New) The method of claim 50, wherein the solid support is a glass bead.--

*Sub D11*  
*Q4*  
~~--52. (New) The method of claim 44, wherein the oligonucleotide (1) is immobilized, (2) comprises the sequence TACGGACAGAACTGTTTTTTTTTTT, (3) is linked to 6-(fluorescein-6-carboxamido) hexanoate at its 5' terminus, and (4) is linked to a C-7 amine at its 3' terminus.--~~

--53. (New) The method of claim 44, wherein the primer in step (B) (1) has the sequence AGGATCAACAACAACCGTA.--

--54. (New) The method of claim 44, wherein the primer in step (B) (2) has the sequence ATCGTCCTGGGCTTTCGCA.--

--55. (New) The method of claim 44, wherein the fluorescent dye which is linked to the primer in step (B) (2) is Texas red.--

--56. (New) The method of claim 44, wherein the sample is a serum sample.--

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~~57. (New) A method for identifying a wildtype human hepatitis~~

~~B virus surface antigen in a sample which comprises:~~

- ~~(A) obtaining viral nucleic acid from a sample;  
(B) amplifying the viral nucleic acid in a polymerase chain reaction using two primers, wherein~~

~~(1) one primer is an oligonucleotide which (i) has a sequence which corresponds to a portion of a nucleic acid which encodes human hepatitis B virus surface antigen, and (ii) is linked at its 5' terminus to a biotin group; and~~

~~(2) the other primer is an oligonucleotide which (1) has a sequence which is complementary to a nucleic acid which encodes human hepatitis B virus surface antigen, and (2) is linked at its 5' terminus to a fluorescent dye;~~

~~(C) obtaining, from the amplified nucleic acid, single stranded nucleic acid which comprises the fluorescent dye;~~

~~(D) contacting the single stranded nucleic acid which comprises the fluorescent dye to an immobilized oligonucleotide, which oligonucleotide comprises a sequence which (1) corresponds to a portion of a wildtype human hepatitis B virus surface antigen nucleic acid, (2) is linked to a fluorescent dye at its 5' terminus; and (3) is linked to a primary amine group at its 3' terminus, under conditions permitting hybridization between the single stranded nucleic acid which comprises the fluorescent dye and the oligonucleotide,~~

Sub D12

04

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~~wherein hybridization between the single stranded  
nucleic acid which comprises the fluorescent dye and the  
oligonucleotide identifies the sample as one containing  
a wildtype human hepatitis B virus surface antigen.--~~

--58. (New) The method of claim 57, wherein the oligonucleotide  
comprises the sequence TACGGACGGAAACTG.--

--59. (New) The method of claim 57, wherein the oligonucleotide  
comprises the sequence TACGGACGGAAACTGTTTTTTTTTTT.--

--60. (New) The method of claim 57, wherein the fluorescent dye  
which is linked to the oligonucleotide is 6-(fluorescein-  
6-carboxamido) hexanoate.--

--61. (New) The method of claim 57, wherein the primary amine  
group which is linked to the oligonucleotide is a C-7  
amine.--

--62. (New) The method of claim 57, wherein the oligonucleotide  
is immobilized on a solid support.--

--63. (New) The method of claim 62, wherein the solid support  
is a glass bead.--

~~--64. (New) The method of claim 57, wherein the oligonucleotide  
(1) is immobilized; (2) comprises the sequence~~